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Discovery and development of Seliciclib. How systems biology approaches can lead to better drug performance

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ABSTRACT

Seliciclib (R-Roscovitine) was identified as an inhibitor of CDKs and has undergone drug development and clinical testing as an anticancer agent. In this review, the authors describe the discovery of Seliciclib and give a brief summary of the biology of the CDKs Seliciclib inhibits. An overview of the published in vitro and in vivo work supporting the development as an anti-cancer agent, from in vitro experiments to animal model studies ending with a summary of the clinical trial results and trials underway is presented. In addition some potential non-oncology applications are explored and the potential mode of action of Seliciclib in these areas is described. Finally the authors argue that optimisation of the therapeutic effects of kinase inhibitors such as Seliciclib could be enhanced using a systems biology approach involving mathematical modelling of the molecular pathways regulating cell growth and division.

1. Introduction

The cell cycle is a fundamental biological process that is tightly regulated by the activity of a series of kinases termed the Cyclin-Dependent Kinases (CDKs). These are such named because of the requirement for binding CDK specific cyclins for their activity (Graña and Reddy, 1995). The activities of these kinases must follow a specific sequence to allow normal cell cycle progression (Morgan, 1997) and abberations in the control of the cell cycle have been linked to a variety of diseases including cancer, inflammatory conditions and neurodegenerative disorders (Zhivotovsky and Orrenius, 2010). The cell cycle proceeds through various checkpoints each of which is regulated by the activity of CDKs that are in turn, regulated by signalling pathways either promoting or inhibiting cell (Chiarle et al., 2001).

The first CDK to be discovered was CDK1, which was originally identified in starfish oocytes as "Maturation Promoting Factor" or MPF. It was found that when oocytes previously arrested in the prophase of the cell cycle, were injected with CDK1, this caused their entry into metaphase, a process known to be associated with protein phosphorylation (Meijer and Guerrier, 1984; Labbé et al., 1989). This observation, that the activity of CDK1, in complex with its partner Cyclin B (CDK1/cyclin B), was required for prophase to metaphase transition, suggested that inhibitors of this kinase could be useful in the treatment of proliferative disorders (Pondaven et al., 1990; Rialet and Meijer, 1991). Supporting this hypothesis, Dimethylaminopurine (DMAP), a drug that was initially identified as a potent inhibitor of mitosis in sea urchins (Rebhun et al., 1973) was subsequently shown to exert its action through inhibition of CDK1/cyclin B complex (Rialet and Meijer, 1991; Neant and Guerrier, 1988). DMAP and a related purine isopentyladenine had *in vitro* IC_{50} values of 120 μM and 55 μM against CDK1/cyclin B respectively. The fact that isopentyadenine was an intermediate in the biosynthesis of the cytokinin group of plant hormones led to a collaboration between Laurent Meijer of the Biological Station in Roscoff and Jaroslav Vesely and Miroslav Strnad at the Institute of Experimental Botany in Olomouc in the Czech Republic. Their collaborative work resulted in the synthesis of a number of substituted purine molecules, the most promising of which was 2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine. This molecule, which was named Olomoucine, was specific in its inhibitory action towards CDK and MAPK (Veselý et al., 1994), an observation which, at the time, was surprising for an ATP analogue. Olomoucine was considerably more potent with an IC_{50} value of 7 μM against CDK1/cyclin B in vitro. Strnad in collaboration with Michel Legraverend of the Institute Marie Curie at Orsay worked together to synthesise more potent and more specific substituted purines, the best of

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Table 1
Studies demonstrating CDK inhibition by Roscovitine in vitro and in vivo.

CDK/Cyclin type	Studied model	Reference
CDK1	Lung cancer cell line	Schutte et al. (1997)
CDK1	Human Colorectal cancer cell line	Abal et al. (2004)
CDK1/Cyclin B	Xenopus oocytes	Meijer et al. (1997)
CDK1/Cyclin B	In vitro kinase assay	Meijer et al. (1997), Raynaud et al. (2005)
CDK1/Cyclin B	In vitro kinase assay	Meijer et al. (1997), Raynaud et al. (2005)
CDK1, CDK2	Human Gastric cell lines	Iseki et al. (1997)
CDK2	Human Pancreatic cell line	Iseki et al. (1998)
CDK2	Human Osteosarcoma, Cervical, Lung carcinoma cell lines	Zhang et al. (2004a,b)
CDK2/Cyclin A, E & B	In vitro kinase assay	Meijer et al. (1997), Havlícek et al. (1997), Biglione et al. (2007), Raynaud et al. (2005)
CDK2/Cyclin B	Mouse lymphocytic leukaemia cell line	Meijer et al. (1997)
CDK2, Cyclin E	In vitro kinase assay, human tumour cell lines, mouse model	McClue et al. (2002)
CDK2/Cyclin B	HCT116 colon cancer cell line	Raynaud et al. (2005)
CDK2/Cyclin D1, Cyclin A2	Human breast cancer cell lines	Nair et al. (2011)
CDK4/Cyclin D1	In vitro kinase assay	Meijer et al. (1997), Raynaud et al. (2005)
CDK4/Cyclin D1	HCT116 colon cancer cell line	Raynaud et al. (2005)
CDK5/P35	In vitro kinase assay	Meijer et al. (1997)
CDK6/Cyclin D3	In vitro kinase assay	Meijer et al. (1997), Raynaud et al. (2005)
CDK7/Cyclin H	Invitro kinase assay	Raynaud et al. (2005)
CDK9/Cyclin T1	In vitro kinase assay & Hela cells	Biglione et al. (2007), Raynaud et al. (2005)

which, 6-(benzylamino)-2(R)-[[1-(hydroxymethyl)propyl]amino]-9-isopropylpurine, termed Roscovitine, had an in vitro IC_{50} value of 0.45 μM against the CDK1/cylin B complex (Havlícek et al., 1997). Roscovitine and olomoucine were subsequently co-crystallised with CDK2 and these structures were used as the basis of molecular models for guiding further medicinal chemistry programmes (De Azevedo et al., 1997).

Roscovitine has been demonstrated to be a potent inhibitor of a number of CDKs including CDK1/cyclin B (0.65 μM), CDK2/cyclin A (0.7 μM), CDK2/cyclin E (0.7 μM), CDK5/p35 (0.2 μM), CDK7/cyclin H (0.49 μM), and CDK9/cyclin H (0.79 μM). However, because Roscovitine is an ATP competitive molecule, the precise IC_{50} values reported vary depending on the concentration of ATP used in the in vitro assay (Wang and Fischer, 2008; Meijer et al., 1997; McClue et al., 2002; Biglione et al., 2007). CDK4/cyclin D1, CDK6/cyclin D3 and over 80 other kinases tested were all insensitive or only weakly inhibited by Roscovitine (Bain et al., 2003, 2007).

As an inhibitor of CDKs 1, 2, 5, 7 and 9 Roscovitine can impact a variety of cellular functions in tissue dependent manner. A summary of studies, demonstrating CDK inhibition by Roscovitine is shown in Table 1. Thus, it is important to have knowledge of individual CDK functions especially while employing a broad spectrum CDK inhibitor. Here, we will briefly examine the biology of different CDKs in an effort to ascertain which therapeutic areas inhibitors of these kinases could impact upon.

As an inhibitor of the CDK family roscovitine can potentially impact upon a number of fundamental processes in cellular biology. Cell division has to be highly regulated and is an area of cellular biology in which the CDK family is heavily involved. Roscovitine-target CDKs 1 and 2 are involved in the control of the transition of cells from G2 to M and G1 to S respectively and as the activity of these kinases is required for initiation and progression of cellular division chemical inhibition of the CDKs has the potential to be useful in proliferative diseases such as cancer.

2. CDK1

CDK1, also referred to as the mitotic kinase, forms a complex with cyclin B (Malumbres and Barbacid, 2007) At 297 amino acids in length and with a molecular weight of 34 kDa its activity is modulated by post-translational modification, being activated or inhibited by site-specific phosphorylation by regulatory kinases including Wee1, Mik1 amd Myt1 on Threonine 161,Tyrosine 15 or Threonine 14 (Schafer, 1998). Hyperactivity of CDK1 either through overexpression of Cyclin B1 or hyperphosphoryation of CDK1 has been observed, observed in several tumours, including breast-, colon- and prostate carcinoma (Pérez de Castro et al., 2007) this supporting the hypothesis that dysregulation of this kinases could cause uncontrolled cellular division.

3. CDK2

Dysregulation of CDK2 activity has also been observed in a variety of malignancies further supporting the theory that inhibition of the CDKs be Roscovitine could be beneficial in the treatment of proliferative diseases. Although CDK2 is a key cell cycle regulator, critical for the transition into the S-phase of the cell cycle, mice lacking the kinase are viable, suggesting that there are other kinases which can compensate for any lack in CDK2 activity (Berthet et al., 2003). CDK2 activity is controlled not just by phosphorylation events by complexation with inhibitory protein partners such as Cip/Kip and of course its cyclin partners Cyclin E and Cyclin A dysregulation of which has been observed in malignancies (Pérez de Castro et al., 2007).

4. CDK5

CDK5 is required for central and peripheral nervous system function (Cruz and Tsai, 2004) and has been implicated in numerous neuronal functions including cytoarchitecture in the brain, neuronal migration, synaptic plasticity, learning and memory and may be involved in the development of neurodegenerative disorders including Alzheimer's and Parkinson's Diseases (Angelo et al., 2006; Cruz and Tsai, 2004; Dhavan and Tsai, 2001). Treatment of the lower eukaryote Dictyostelium discoideum with Roscovitine led to an inhibition not only of the single-cell growth phase of the organism but also arrested translocation of the protein between the nucleus and cytoplasm raising the possibility that at least part of the biological effects of Roscovitine may be due to secondary effects such as protein location (Huber and O'Day, 2012). Inhibition of CDK5 in lower eukaryotes has also indicated a role for the kinase in development, cytoskeletal organisation and calcium channel function (Huber and O'Day, 2012; Prithviraj et al., 2012; Wen et al., 2013). CDK5 has also been implicated in modulating the metastatic potential of breast and prostate carcinomas (Goodyear and Sharma, 2007; Strock et al., 2006). It is unusual in that it exhibits kinase activity only when bound to non-cyclin activators CDK5R1 and CDK5R2 although structural studies on these proteins have shown structural similarity with the cyclins (Cheung and Ip, 2012).

These observations that CDK5, a kinase that is inhibited by Roscovitine broaden the therapeutic applications of the compound further beyond the less well documented areas of proliferative disease and virology. The potential of Roscovitine to treat neurological disorders such as Alzheimers and Parkinson's is very exciting given the paucity of treatments currently available for these treatments

and its low toxicity and excellent tolerability are surely plus points in this therapeutic area.

5. CDK7

CDK7 binds not only to its cyclin partner Cyclin H but also forms a trimer with a third partner, MAT1. Termed the Cyclin-dependent kinase Activating Kinase or CAK this trimer phosphorylates CDKs 1, 2, 4 and 6 on their key activating residues (Lolli and Johnson, 2005). A role in cell division has been observed in some eukaryotic systems including yeast, where loss of activity causes cell cycle arrest and drosophila in which mutations are lethal before or during pupation (Larochelle et al., 1998; Wallenfang and Seydoux, 2002). In mammalian cells loss of MAT1 induces cellular arrest in G1 and cell death by apoptosis (Wu et al., 1999). The cell-cycle role of CDK7 in cell death is less clear cut than some of the other CDKs due to the fact it is also involved in the control of transcriptional. CAK forms part of the large multimeric general transcription factor TFIIH where it phosphorylates the C-Terminal Domain (CTD) of RNA Polymerase II improving the efficiency of transcriptional initiation and elongation (Maldonado and Reinberg, 1995). As the kinase has multiple biological effects it is more difficult to define unambiguously which inhibition causes which effect.

6. CDK9

CDK9 with its partner Cyclins T or K also forms part of the transcriptional machinery being a core part of the multi-subunit positive transcription elongation factor b (p-TEFb) (Loyer et al., 2005; Malumbres and Barbacid, 2005; Romano and Giordano, 2008; Yu and Cortez, 2011) which is involved in improving the transcriptional elongation from RNA Pol II dependent promoters. This class of promoter drives expression of multiple key developmental and cellular response genes as well as the majority of protein encoding genes (Nechaev and Adelman, 2011).

The discovery of role of the CDKs 7 and 9 in the control of gene transcription opened up new possibilities for roscovitine in new therapeutic areas, most importantly in virology where the importance of their activity has been recognised in the replication of Herpes Simplex Virus, Human Imunodeficiency Virus and Human Cytomegalovirus (Boeing et al., 2010; Durand and Roizman, 2008; Schang et al., 1998; Yang et al., 1997)

7. In vitro studies of Roscovitine as an anti cancer drug

Although CDKs play pivotal roles in a range of cellular functions, studies with Roscovitine have focussed largely on its inhibitory effects on cell cycle progression, mainly with a view to its development as a potential anti-cancer agent. Roscovitine has been tested on more than 100 cell lines, including the NCI-60 panel of the United States' National Cancer Institute (Shoemaker, 2006).

In 1997, Meijer et al. showed that constant exposure to Roscovitine over a 48 h period inhibited the growth of 60 different cell lines from 9 different tissue types when compared with non-exposed cells. The average IC_{50} across all cell lines was 16 μM (Meijer et al., 1997). In a separate study Raynaud and colleagues reported that Roscovitine inhibited the growth of 24 cell lines with an average IC_{50} value of 14.6 μM (Raynaud et al., 2005). Other studies have shown that in the mouse leukaemia cell line L1210, Roscovitine led to an accumulation of the cells in G2/M cycle. This accumulation of cells in the G2/M phase was also observed in A549 human lung cancer cell lines in a detailed study by McClue et al. (2002). This group demonstrated that 24 h of treatment with Roscovitine led to a significant increase in apoptosis. Schutte and colleagues examined the effects of both roscovitine and olomoucine on the kinetics of the cell cycle in the human lung cancer cell line MR65 and the neuroblastoma line CHP212 (Schutte et al., 1997). In this study, cells exposed to either of the compounds showed delays in the transitions from G1 to S phase and from G2/M to G1 as well as a prolonged S phase. They also observed changes in cell morphology that were indicative of apoptosis in the treated cells. In a study using normal human fibroblasts, Alessi et al. reported a reversible block in G1 after Roscovitine or Olomoucine treatments (Alessi et al., 1998) and reduced levels of hyper-phosphorylated Rb, indicating cell cycle arrest, but unchanged levels of Proliferating Cell Nuclear Antigen (PCNA) and Cyclins D1 and E. In another study, treatment of human gastric cell lines SIIA, AGS, MKN45-630 and SNU-1, resulted in an increase in the proportion of cells in G2/M and S phases. In SIIA cells, treatment led to a reduction in levels of phosphorylated Histone H1, suggesting that the compound was inhibiting CDK1 and CDK2 (Iseki et al., 1997). A year later, same group also examined four human pancreatic cell lines with differing genetic lesions and showed that Roscovitine and Olomoucine inhibited CDK2 activity and cellular proliferation independent of the p53, K-Ras or p16 status (Iseki et al., 1998). During the same year, Mgbonyebi and colleagues investigated the effect of Roscovitine on the proliferation of immortal and neoplastic breast cancer cells and reported that Oestrogen Receptor (ER) positive and ER negative cell lines were sensitive to Roscovitine (Mgbonyebi et al., 1998). In a further study, the same group reported that treatment of ER-ve MD-MB-231 cells with Roscovitine for between 1 and 10 days induced morphological changes in the cells consistent with the induction of apoptosis (Mgbonyebi et al., 1999).

Responses to Roscovitine have also been investigated in combination with a number of other chemotherapeutic agents in vitro. It has been shown to have potential synergistic relationships with camptothecin in the breast tumour line MCF7, the histone deacetylase inhibitor LAQ824 in leukaemic cell lines HL60, with doxorubicin in sarcoma cell lines and also with irinotecan in a p53-mutated colon cancer (Lu et al., 2001; Lambert et al., 2008; Abal et al., 2004).

We have previously studied the effects of R-Roscovitine (CYC202) on the physiology of normal and transformed human cells. These studies revealed for the first time that at therapeutic doses, the drug is not toxic to normal keratinocytes, but at higher doses CYC202 can affect components of major signalling pathways, (e.g. p38), highlighting potential side-effects of the drug in vivo (Atanasova et al., 2005, 2007). In addition to the induction of apoptosis and cell cycle effects, Roscovitine has been reported to inhibit DNA synthesis in primary human glioma samples by almost 90% (Yakisich et al., 1999) as well as inducing mucinous differentiation in the human non-small cell lung cancer line NCI-H348 (Lee et al., 1999).

In summary Roscovitine has been reported to induce apoptosis in several cell lines independently of p53 status. Cell death has been detected in all phases of the cell cycle via a variety of potential mechanisms including inhibition of the cell cycle and effects on transcription due to reduced phosphorylation of the CTD of RNA polymerase II by CDK7 and CDK9 (Wesierska-Gadek et al., 2005, 2008). However, treatment with Roscovitine had relatively little impact on global transcription with only a small number of transcripts found to be significantly reduced. It is worth noting that those proteins whose transcript level was found to be reduced by Roscovitine treatment were mostly pro-survival factors on which tumour cells may be more dependent than normal cells (Meijer and Galons, 2006). These observations suggest that cell death induced by Roscovitine may be due to the reduction in levels of a small number of survival factors such as Mcl-1, XIAP and survivin (Lacrima et al., 2005; Mohapatra et al., 2005).

8. In vivo studies of Roscovitine as an anti cancer drug

Roscovitine has been tested extensively in animal models, largely in xenograft models of various cancers, as part of its development as an anticancer agent. In addition it has been examined in all the standard toxicology tests required by regulatory authorities and although the authors are not aware of any toxicological issues, that data will not be discussed in this review.

In the xenograft models tested, Roscovitine has generally led to reductions in tumour growth rather than absolute reductions in tumour volume. The compound has been dosed both by oral gavage as well as by intra-peritoneal injection and a variety of dosing schedules have been employed in the studies. In a report originally published in 2002, McClue et al. studied mice bearing tumours derived from human uterine cell line MES-SA/Dx5 or the human colon cell line LOVO, both cell lines being sensitive to Roscovitine in in vitro studies (McClue et al., 2002). Mice with established LOVO tumours were treated with Roscovitine at a dose of 100 mg/kg by intra-peritoneal injection three times per day for 5 days. Tumour volume was observed for 32 days following the initiation of treatment, over which period Roscovitine treated mice showed 55% of the tumour burden when compared to untreated control mice (McClue et al., 2002). Mice bearing MES-SA/Dx5 derived tumours were dosed intra-peritoneally three times per day either at a lower dose of 200 mg/kg for 10 days or higher dose of 500 mg/kg for 3 days. In both of the treatment regimes, tumour volumes were reduced compared to untreated control mice, to 65% of control at 200 mg/kg and to 38% of control in the mice treated at 500 mg/kg (McClue et al., 2002).

The most striking result with xenografts models using Roscovitine as a single agent was seen in mice bearing A4573 Ewings Sarcoma derived tumours. In this study, mice dosed with Roscovitine once per day by intra-peritoneal injection at a dose of 50 mg/kg for 5 days showed an 85% reduction in tumour burden compared to untreated mice at the end of the dosing period (Tirado et al., 2005). Other studies have examined the effects of Roscovitine on mice bearing tumours of colon, lung, brain, breast and nasopharangeal origin. The effects of Roscovitine in these studies have slowed tumour growth resulting in reductions in tumour volume relative to control animals but has failed to cause reductions in absolute tumour volume (Cheng et al., 2012; Fleming et al., 2008; Hui et al., 2009; Nair et al., 2011; Raynaud et al., 2005).

Consistent with in vitro studies, Roscovitine has also been tested in combination with other anticancer agents or treatments in in vivo studies. Most impressive was a combination of Roscovitine with ionising radiation for the treatment of mice bearing tumours from two Epstein–Barr Virus (EBV) positive cell lines derived from nasopharangeal cancer patients (Hui et al., 2009). In this study, mice were dosed with Roscovitine by intra-peritoneal injection twice per day for 5 days followed by a break for two days and then 5 further days of Roscovitine dosing. During dosing period, the mice were treated twice with 6 Gy of ionising radiation. In a 30 day period following such treatments, treated mice developed only 20% of the tumour burden compared to untreated mice (Hui et al., 2009).

Fleming et al. examined the effects of a combination of Roscovitine and Erlotinib (a reversible Epidermal Growth Factor Receptor (EGFR) inhibitor) on tumour growth in mice bearing H358 non-small cell lung cancer tumours (Fleming et al., 2008). In a 28 day treatment window, Erlotinib was dosed orally every day at 100 mg/kg and Roscovitine was dosed twice per day by intra-peritoneal injection at 50 mg/kg on a 5 day on/2 day off schedule. Seven weeks after the initiation of treatment, tumour volume in the treated animals was just 7% of that in the untreated animals. In the same study Roscovitine alone showed no significant reduction in tumour growth and Erlotinib alone reduced tumour growth by only 56% clearly showing that these two compounds act synergistically to reduce tumour growth (Fleming et al., 2008).

In another study, Roscovitine was tested in a xenograft GBM43 glioma model in combination with an experimental PI-3 kinase inhibitor, PIK-90 (Cheng et al., 2012). Both drugs were dosed intraperitoneally four times per day for 12 days, Roscovitine at 50 mg/kg and PIK-90 at 40 mg/kg. At the end of the 12-day treatment period, mice treated with the combination of the drugs showed 75% reduction in tumour volume as compared to that in untreated animals. Treatment with Roscovitine or PIK-90 alone reduced tumour volumes by approximately 40% and 50% respectively indicating that the combination of the two drugs was not more than additive (Cheng et al., 2012).

We have identified the specific CDK inhibitor, p27 and its substrate Rb, as biomarkers for CYC202 mediated cell growth inhibition, and demonstrated its usefulness for monitoring inhibition of its major target (CDK2) in cellulo and in vivo (Whittaker et al., 2001; McClue et al., 2002; Zhang et al., 2004a).

In summary, the in vivo effects of Roscovitine as a single agent for the treatment of xenograft models of cancer are mild. The great advantage of the drug is that treatment is well tolerated making it ideal for use in combination with other drugs or treatments where tumour burden could be significantly reduced while still remaining well tolerated. Hence, it seems to the authors that the future of Roscovitine in cancer will be its use in combination with other anticancer agents.

9. Clinical studies using Roscovitine as an anticancer agent

Roscovitine (generic name Seliciclib) has been tested in a number of Phase I and II clinical trials sponsored by the biopharmaceutical company Cyclacel Pharmaceuticals Inc. The drug has been used to treat around 450 cancer patients and has shown a degree of anticancer activity in around half of those patients. In an initial Phase I trial with patients suffering from refractory solid tumours, a schedule of oral dosing of twice per day for 7 days in a 21-day cycle was used. A maximum tolerated dose of 800 mg with dose limiting toxicities of fatigue, rash, hyponatremia and hypercalcemia was reached. Although no tumour reductions were observed, stable disease was recorded in 8 patients (Benson et al., 2007). A subsequent Phase I trial in cancer patients was carried out using different dosing schedule and reached a maximum tolerated dose of 1800 mg twice per day when dosing twice daily for 3 days in a 2 week cycle. One hepatocellular cancer patient had a partial response to treatment and others had periods of stable disease. Dose limiting toxicities were similar to those observed in the initial trial (Le Tourneau et al., 2010). Roscovitine has also been tested in a Phase I trial in combination with Gemcitabine and Cisplatin in non-small cell lung cancer patients. The maximum tolerated dose of Roscovitine was 800 mg in combination with 1000 mg/m^2 Gemcitabine and 75 mg/m^2 Cisplatin. Interestingly, for this drug combination the level of haematological toxicity observed was relatively low (Siegel-Lakhai et al., 2005).

Interim results from a Phase I trial of patients with nasopharangeal and other solid tumours, 7 of the 10 patients with nasopharangeal tumours and 4 of the 13 patients with other solid tumours had stable disease while on trial. Dosing in this trial was orally administered at either 400 mg or 800 mg of Roscovitine for 4 days in a 2-week cycle. Both dosing regimens were considered tolerable with results being positive enough to warrant further investigation in a randomised trial of patients with nasopharangeal cancer (NPC) (Yeo et al., 2009). Another NPC study reported that half of the evaluable patients showed signs of a reduction in tumour volume and tumour biopsies from before and after treatment

suggesting that Roscovitine was inducing apoptosis and necrosis in the tumours (Hsieh et al., 2009).

Roscovitine has been evaluated in a Phase II trial in 187 patients suffering with Non-Small Cell Lung Cancer (NSCLC). The trial failed to meet the primary endpoint of improving progression free survival but patients treated with the drug did show longer median survival (Cyclacel, 2010). Roscovitine is also undergoing clinical testing as a combination treatment together with nucleoside analogue Sapacitabine (CYC682) in patients with advanced solid tumours (Cyclacel, 2013a), as well as in combination with EGFR inhibitor Erlotininb (Tarceva) in patients with advanced solid tumours markers (Cyclacel, 2008) and finally in the treatment of patients with rheumatoid arthritis, who have not responded to current conventional treatments (Cyclacel, 2013b).

10. Beyond cancer; potential of Roscovitine as a therapeutic drug for other diseases

10.1. Kidney disease

Polycystic kidney disease (PKD) is one of the most common life-threatening genetic diseases affecting some 12.5 million people across the globe (Anon, 2014). The condition is normally inherited in a dominant Mendelian manner, but can also be passed on recessively, and causes the formation of multiple, fluid-filled cysts in the kidneys. This leads to massive enlargement of kidneys and in function (Martinez and Grantham, 1995). Mutations in PKD genes are thought to disrupt localisation of ion channels and growth factor receptors and lead to fluid retention and cellular proliferation in the kidney. This increased renal cell proliferation has been targeted using roscovitine, an approach that has met with some success in mouse models of both indolent (jck) and aggressive (cpk) forms of the disease (Bukanov et al., 2006). Treatment with Roscovitine inhibited disease progression and improved renal function. Renal cells from treated mice showed reduced levels of phosphorylation on both Rb and Cyclin D, observations consistent with a block at the G1/S phase of the cell cycle (Bukanov et al., 2006). Roscovitine has been examined in a number of animal models of various varieties of Glomerulonephritis and has been shown to help prevent or improve pre-existing disease and demonstrated reductions in inflammatory markers associated with the disease (Milovanceva-Popovska et al., 2005; Pippin et al., 1997; Sheryanna et al., 2011; Zoja et al., 2007).

10.2. Potential of Roscovitine to combat viral infections

The use of Roscovitine to combat viral infections is based on the rationale that viruses, such as papilloma- or adeno-virus, can replicate only in dividing cells and hence show a requirement for cellular CDK activity to drive the cell into and through the phase of the cell cycle. As such, inhibition of host cell's CDK activity may cause cessation of viral replication. Viruses, such as Human Immunodeficiency Virus (HIV) and Herpes Simplex Virus (HSV), which can replicate in non-dividing cells, have a less obvious requirement for CDK activity, although some studies have revealed that, at least in vitro, replication of many of these viruses can be inhibited roscovitine. Human Cytomegalovirus (HCMV) infection in healthy individuals is rare despite exposure being common. However in immune compromised patients, infection with HCMV is a significant issue and leads to increased levels of morbidity and mortality. Infection acts on cells and create an environment favourable to viral replication that can be disrupted by small molecule ATP mimics such as Roscovitine. In one such study, treatment of HCMV infected cells with Roscovitine reduced viral replication possibly by reducing phosphorylation of the key viral regulatory protein pUL69 by CDK9/cyclin T1 and disruption of its localisation (Rechter et al., 2009; Sanchez et al., 2003; Schang et al., 2006).

Efficient Human Immunodeficiency Virus-1 (HIV -1) gene expression relies on RNA Pol II CTD phosphorylating activity of CDK7 (Boeing et al., 2010) and CDK9 (Yang et al., 1997). Furthermore, cells latently infected with HIV-1 lose the proteinaceous CDK inhibitor p21/waf1 leading to increased CDK2/cyclin E activity (Clark et al., 2000). These findings clearly provided a rationale for employing Roscovitine based therapies, which could theoretically have the potential to be an effective anti-HIV-1 agent. Indeed, In vitro Roscovitine treatment demonstrated reduction in viral titre of both wild-type and drug resistant strains of HIV-1 and with an induction in apoptosis in T-cells, monocytes and PBMCs irrespective of the phase of the cell cycle (Agbottah et al., 2005).

Although Herpes Simplex Viruses (HSVs) encode protein kinases within their genome, many still rely on host cell kinase activity for successful replication. Consistent with this CDK9 has been reported to enhance viral transcription by phosphorylating the CTD of RNA Pol II (Durand and Roizman, 2008). Roscovitine and Olomoucine have been shown to inhibit HSV replication via a non-cell cycle blocking mechanism (Schang et al., 1998, 1999).

10.3. Roscovitine as an anti-inflammatory drug

Roscovitine has recently been explored for its potential as an anti inflammatory agent. This is due to the observation that CDK inhibitors such as Roscovitine can induce neutrophil apoptosis as well as block lymphocyte proliferation (Leitch et al., 2009). Rossi et al. examined the effects of Roscovitine in a mouse carrageenan-induced pleurisy model and a mouse bleomycin-induced lung injury model. Their results showed that Roscovitine caused a reduction in oedema, levels of inflammatory markers and increased survival of treated mice relative to controls (Rossi et al., 2006). Furthermore, Leitch and colleagues have shown that the anti-inflammatory action of Roscovitine in neutrophils is due to a reduction in RNA Pol II transcription and induction of apoptosis caused by inhibition of CDKs 7 and 9 (Leitch et al., 2012).

Pneumonia is a lung disease that kills about 4 million people per year worldwide and is typically caused by bacterial or viral infection. Streptococcus pneumonia is a common causative agent whose cell wall contains the pro-inflammatory molecule lipoteichoic acid (LTA). This chemical induces release of reactive oxygen, hydrolases, proteases, growth factors and cytotoxic cytokines from macrophages and neutrophils. It has been found that treatment of alveolar macrophages and respiratory epithelial cell lines with Roscovitine following exposure to LTA led to a reduction in the secretion of TNF-α and keratinocyte chemoattractant (KC) (Hoogendijk et al., 2012). In the same study it was also shown that Roscovitine reduced the number of Polymorphonuclear leukocytes in the lungs of mice with LTS-induced inflammation (Hoogendijk et al., 2012).

10.4. Roscovitine in the prevention of ischaemia/stroke induced tissue damage

Ischaemic strokes cause tissue damage and loss of function to parts of the brain when blood flow to these areas is reduced. S-Roscovitine has been tested in models of ischaemia and was shown to cross the blood:brain barrier. Furthermore, it caused a reduction in CDK5/p25 activity and reduced brain damage when dosed after an experimentally induced ischaemic episode (Menn et al., 2010).

During transplant or bypass surgery, stroke or myocardial infarction tissue is starved of blood flow, and hence oxygen, before flow is returned. This transient ischaemia can induce inflammation and oxidative stress and cause damage to the organ when blood flow is reinstated (Clavien et al., 1992; Langdale et al., 1993).

In response to previously reported anti-inflammatory effects of Roscovitine, Aydemir and colleagues tested it in a rat model of renal ischaemia/reperfusion (IR). Upon treatment with Roscovitine, followed by assessment of circulating biomarkers and histopathological examination, the authors concluded that there was less renal damage in the disease model as compared to the untreated counterparts (Aydemir et al., 2002). Topaloglu et al. also concluded that pre-treatment with Roscovitine reduced the number of dead cells, apoptotic cells and leucocyte infiltration in the livers of rats that had induced-IR to the right hepatic lobe, consistent with a protective effect and reduced inflammation (Topaloglu et al., 2003).

10.5. Roscovitine in anti fibrotic therapy

Scleroderma is a condition in which excess connective tissue is formed either cutaneously or systemically leading to changes in the vasculature. Roscovitine has been shown by Steinman and colleagues to reduce expression of collagen, fibronectin and connective tissue growth factor (CTGF) in systemic sclerosis fibroblasts. This was owing to the ability of Roscovitine to cause a reduction in transcription of the genes, an effect that could not be reversed by treatment with pro-fibrotic cytokines (Steinman et al., 2012). Given the lack of current therapies for Scleroderma, these observations certainly support further investigation and advocate the importance of Roscovitine as a therapy option for fibrosis

10.6. Roscovitine in glaucoma

Glaucoma is a term used to describe a number of eye disorders caused by changes in intraocular pressure, most commonly associated with increased intraocular pressure. If left untreated, glaucoma can lead to irreversible retinal damage and blindness. Both R- and S-Roscovitine have been evaluated in a rabbit glaucoma model and have been shown to reduce intra-ocular pressure (Kasai et al., 2013).

10.7. Roscovitine in controlling seizures

Paroxysmal attacks are short seizures that are associated with other disorders including multiple sclerosis, head injury, stroke and epilepsy amongst others (Anon, 2007). Gamma Amino Butyric Acid (GABA) is a mammalian neurotransmitter and plays a key role in the control of neuron excitability by binding and altering activity of its GABA receptor. Small molecules, such as Roscovitine, have also been shown to alter GABA receptor activity and thus represent an avenue that could be of potential benefit in the treatment of neurological conditions such as epilepsy. Ivanov et al. have shown that Roscovitine increases GABA mediated current in rat hippocampal neurons without modifying $GABA_A$ receptors and suppresses "spiking" in hippocampal pyramidal cells. This activity may ultimately be of benefit in the treatment of paroxysmal activity and is certainly worthy of further research (Ivanov et al., 2008).

10.8. Roscovitine in the treatment of neurodegenerative diseases

Given the association of aberrant CDK5 activity with neurological conditions such as Alzheimer's, Parkinson's, Niemann Pick Type C (NPC) and Amyotrophic Lateral Sclerosis (ALS). roscovitine has been tested in some disease-relevant animal models (Kusakawa et al., 2000; Hung et al., 2005; Lopes et al., 2007).

Zhang et al. have demonstrated that roscovitine decreased phosphorylation of tau and other neurofilament and mitotic proteins when dosed via the intracerebroventricular route in a mouse model of Niemann-Pick Type C disease. Importantly this effect on markers of CDK5 activity was accompanied by an improvement in motor defects typical in the condition and also had effects on Purkinje neuron lifespan and the formation of axonal spheroids (Zhang et al., 2004b).

In a rat model of Parkinson's Disease (PD) Chagniel and colleagues investigated the effect of roscovitine and calpain inhibitors on abnormal involuntary movements (AIMS) associated with the condition. Intrastriatal infusion of roscovitine reduced the severity and amplitude of AIMS as well as reversing biomarkers associated with L-DOPA-induced dyskinesia (LID). The effect on the symptoms of PD by CDK5 inhibition by roscovitine was less marked than for the calpain inhibitor suggesting that inhibition in the formation of CDK5/p25 is more effective than inhibiting the aberrantly activated kinase itself (Chagniel et al., 2012).

Despite the evidence that CDK5 activity is implicated in the aetiology of Alzheimer's disease Sadleir and Vassar have reported that treatment of primary neurons with roscovitine could cause elevation of the β-secretase enzyme BACE-1 that initiates the formation of amyloid-β peptide that comprises amyloid plaques in the brains of sufferers, a potentially negative effect (Sadleir and Vassar, 2012).

10.9. Roscovitine treatment in preventing cardiac hypertrophy

Cardiac hypertrophy may represent a physiological or pathological condition in which cardiac myocytes expand in size giving rise to a hypertrophic heart. While the onset of heart hypertrophy may represent heart's physiological requirement in order to meet the blood pumping overload, persistent hypertrophic condition may cause detrimental effects on tissue and permanent damage (Krystof et al., 2010). The principle behind employing a Roscovitine-based therapy to prevent hypertrophy is that hypertrophic cells show elevated levels of transcription and translation demonstrating hyperactive CDK's, especially CDK9 that is involved in activation of RNA pol-II (Trifonov et al., 2013). To investigate drug effects on cardiovascular physiology, we firstly developed a 'mini-hearts' assay, consisting of organ cultures of human stem cell-derived cardiomyocytes. Using the organ culture model, our studies showed no evidence of any treatment-related effects on cardiomyocyte physiology. We then induced hypertrophic condition in our organ culture and finally demonstrated that R-Roscovitine (CYC-202) was able to prevent development of heart hypertrophy in vitro in vitro (Zhelev et al., 2013a,b).

The above examples serve to demonstrate the promise of small molecule inhibitors, such as Roscovitine, in combating variety of disease phenotypes owing to the multifaceted roles of their targets; CDKs. However, the same also presents a challenge because of the general involvement of CDK function in numerous essential biochemical pathways. This not only mandates achieving specificity in action of the drug towards its target itself, but also in inhibition of its target in context dependent manner. This might warrant devising treatment regimes selective for their action in either specific tissue types, in cell cycle dependent manner or in threshold dependent manner (e.g. only inhibiting CDK with activity beyond a certain threshold). We believe that owing to the ubiquitous nature of CDK function, and the associated alteration of the complex biochemical signalling cascades resulting from their inhibition, a systematic approach is required that study pathway components globally. This warrants a Systems Biology approach for pathway analysis (next section).

11. Role of systems biology in drug discovery and development

Over the last quarter of a century new techniques have allowed biological scientists to capture increasing quantities of data. Initially high-throughput screening (HTS) allowed scientists

to automate the testing of libraries of hundreds of thousands of different molecules allowing the identification of molecules that modulated very specific biological processes. In parallel the development of microarray technology quickly allowed biological scientists to measure the effect of a stimulus on the expression of all 30,000 plus genes in a cell simultaneously. More recently developments in next generation sequencing (NGS), proteomics and metabolomics have allowed scientists to rapidly sequence all 3 billion base pairs of the human genome and identify post-translational changes in proteins and in the levels of cellular metabolites on a scale that was unimaginable just a generation ago.

The massive increase in data available has increased the pressure to devise novel methods of interpreting the massive quantities of data now available. The new techniques have developed methods in isolation for analysing the data produced, for example in HTS robust methods now exist for the identification of the molecules which modulate a biological process and likewise with microarray experimentation, data analysis is robust in identifying the transcripts whose expression has been altered by a biological stimulus. The great challenge for the next generation of data scientists is to devise methods to analyse and cross-reference all the data from all the newly developed technologies and to allow its use in a truly integrated manner (Clyde et al., 2011). The biological sciences industry and allied industries such as the pharmaceutical have driven the development of these technologies and have invested billions of dollars to date but still the fruit of these labours are still to be seen in the development of new drugs which according to FDA statistics have declined over the period in which these new technologies have appeared (FDA, 2013)

Systems biology is a powerful tool and its utilisation in drug discovery and design is an exciting and encouraging development (Clyde et al., 2006). The intrinsic properties and features of a systems biology approach make it particularly suitable for integration into the process of drug discovery and design. Firstly, by definition, systems biology is the quantitative characterisation of complex interrelationships of biological systems and how they communicate and interact to bring about a biological change. Following the initial steps of qualitative characterisation, the process of drug discovery requires establishment of multiple quantitative parameters, e.g. half life of drug, drug dose and time dependency, degree of inhibition and potency, all of which require quantitative analyses that form the basis of the systems biology approach. Secondly, characterisation of off-target effects of drugs is a central criterion on which drug effectiveness and efficacy is founded. System biology involves the analysis of the whole biological network of a given process. A key feature of dynamic modelling of biochemical pathways through systems biology is sensitivity analysis. This not only determines and underpins key nodes in the network of signalling which are critical to form the functional module, but also links that overall functional module with individual components of network (Lebedeva et al., 2012; Idowu et al., 2011). Such information could be vital for drug discovery process for the characterisation of off target effects and the overall effect on physiology at the cellular and the whole organism level. Third is the growing realisation of the requirement for a holistic approach to drug discovery and for identifying druggable targets. This is due to the fact that the reductionist approach defined earlier for simpler systems (one protein, one function), does not take into account a fundamental property of biological systems, manifested as emergent behaviour. This emergent behaviour could only be accounted for through systems biology approach, which parameterises a particular biological process as a whole. This feature makes systems biology a vital tool for drug development and target validation. Furthermore, drugs based on simple, reductionist models limit the scope and space for wider target identification and unexpected effects, which using the global approach intrinsic to systems biology could be avoided. The fourth aspect is related to economic and social implications. Using molecular modelling approaches, the early in silico determination of the properties of novel drugs could eliminate some aspects of biological testing. Robust predictive in silico modelling, based on many chemical structures, of half life and drug retention times, would be cheaper and could avoid or reduce animal and perhaps human testing.

As mentioned above, technological advancements and high throughput technologies have shifted the bottleneck from data generation to data interpretation. Mathematical modelling of complex biological systems and systems biology has previously exposed features of signalling not obvious from biological analysis alone (Idowu et al., 2013). This will be instrumental in delivering the benefits of employing systems biology in the drug discovery domain as scientists realise that the primary drug targets could be quite remote from the immediate signalling network implicated in a disease phenotype. Moreover, unless a drug candidate is absolutely specific, some degree of predictive power will be provided only if a systems biology approach is utilised.

An integrated approach for drug discovery via systems analysis could allow for the deciphering of complex biological networks and generating novel hypotheses. These hypotheses could present themselves as new avenues of intervention within a network of signalling complex for lead compound development.

The development of systems protocols is a stepwise process and should allow for directed evolution. A prerequisite for initial development is not just large amount of relevant quantitative data (e.g. the -omics approach), but best approximations of contextual information of the disease, e.g. Cancer microenvironment, disease history and therapy history etc. Ideally, such information should be derived from tissues for which data from non-diseased tissue also exists. The initial steps will involve bioinformatics and statistical analyses to establish correlations and propose cause and affect relationships. Statistical protocols will then need to be developed based on which, after filtering information for relevance such as biological context and experimental setting, a priori disease knowledge be must incorporated. A framework and network template of the topology of the disease could then be developed via computer simulation with the input of biological insights from experts operating within relevant biological domains. Each node in the network would represent a quantifiable biological variable, e.g. up- or down-regulation of expression, differential localisation, post-translational modification, protein–protein interaction, degradation and so on, and inter-relationships between different nodes could be developed making use of temporal data and novel information from biological experimentation. At this point, the model may suggest development of novel cell based assays that provide data on a particular signalling aspect that could independently confirm and validate some of the assumptions of the model (Tummala et al., 2012). Different functional modules could then be identified within the wider network and connections between individual modules established.

Once such a framework is developed, it could be used for sensitivity analysis for each node, accelerate hypothesis generation and computationally fine-tune the functional modules for exploratory purposes and to achieve specificity towards diseased phenotypes and comparison with the normal phenotype. The predictive network simulation model could then propose specific intervention strategies and short list possible druggable targets in the whole network. In silico manipulation of different functional centres within the network could be performed and the resulting signalling responses and compensatory pathways that could either resist or accentuate any responses to intervention could be identified and examined. This would be particularly useful as many growth signalling pathways may respond unexpectedly to novel drugs (Goltsov et al., 2014a,b) A global systems analysis, one that

is developed through systems biology, may highlight any such compensatory cancer specific pathways that emerge and suggest alternative or multiple drug targets (Goltsov et al., 2014a,b). Such systems biology protocols for drug development could not just aid in drug target identification, but also devise and inform treatment regimes, e.g. monotherapy vs. combination therapy, or simultaneous vs. sequential combination therapy or full inhibition vs. partial inhibition etc. (Khalil et al., 2012; Tummala et al., 2012; Zhelev et al., 2014).

Conflict of interest

No conflict of interest is declared by any of the authors.

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